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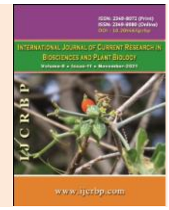

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Evaluation of some heavy metal residues content in hen's table eggs marketed in Egypt

Maria Ahmed El-Ansary *

Food Control Department, Faculty of Veterinary Medicine, Alexandria University, Egypt

*Corresponding author; e-mail: mariaelansary@yahoo.com

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Abstract

In the present study, 120 fresh hen's egg samples (Balady and farm) were randomly collected from supermarkets of Alexandria and El-Behera Governorates, Egypt and analyzed for lead and Cadmium residues expressed in (µg kg^{-1}) in egg content using atomic absorption Spectrophotometer device. Results revealed that the total mean concentrations of Cd was 0.23± 0.0122 (µg kg^{-1}) and the total mean concentration of lead was 123.45 ± 14.597(µg kg^{-1}). Results also revealed that the residues of Cadmium in all examined samples were within the permissible limit (0.05mg kg^{-1}) and lead (Pb) concentrations exceeded the MRL value of (0.1 mg kg^{-1}) in 7/120 (5.83%) of the total examined eggs. Lead contamination of hen's eggs represents a potential public health hazard, especially to children repeatedly consuming eggs from contaminated hen's eggs. The public health hazard and adverse outcomes of the examined toxic heavy metals on human consumers were discussed. Appropriate precautions are warranted to minimize the heavy metal consumption contamination especially of lead.

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Introduction

Eggs are among the most nutritious foods on earth and can be part of a healthy diet. In Egypt, eggs are one of the important protein resources. Eggs represent about 1.32 % of the total daily protein for an individual as sources of animal protein in Egypt. In 2016 Annual per capita consumption of egg was 94 eggs/ capita/ year (CAPMAS, 2018) in Egypt. Eggs are generally consumed by different income classes, and contamination of eggs can constitute problems to the general population. Hen's eggs could be contaminated by toxic heavy metals due to industrial waste, geochemical structures and agricultural activities is a serious problem for environmental and human health. Birds have served as bio indicator for a number of environmental contaminants predominantly heavy metals (O'Connell and Jackson, 2000). Heavy metals can be transmitted from poultry to eggs through environmental pollution or via the food chain (Giri and Singh, 2019). Some of the heavy metals that are known as potentially toxic include cadmium and lead; toxic elements can be harmful even at low concentrations when ingested over a long period of time. Cd and Pb toxic heavy metals when ingested in sufficient quantities, has been associated with adverse human health effects, potentially including carcinogenesis, neurotoxicity, nephrotoxicity and reproductive issues (Wallace et al., 2020).

Generally, increasing the intake of toxic elements poses hazards to both animals and humans. It is therefore necessary to estimate the risks associated with the regular consumption of such eggs as well as the need for more elaborate studies to estimate their impact in respect to possible health effects. Therefore, the present study was considered in order to assess the level of two elements recognized as toxic heavy metals Cd, and Pb, in the hen's eggs from different locations in Alexandria and El-Behera Governorates, Egypt with a view of providing information about the risk associated with their consumption

Materials and methods

Sampling

One hundred and twenty eggs of local hens (sixty each of Balady and farm eggs) were collected from two sectors representing two geographic areas in Egypt. These sectors were: Alexandria Governorate and El-Behera Governorate; sixty eggs (30 Balady and 30 farm eggs) were purchased from the local market in each Governorate.

Egg preparation and analysis

To analyze the metals in egg content according to method previously described by AOAC, (2005) the eggs were washed vigorously with de ionized water, then opened carefully; by cut in the air cell end using pointed forceps and dissecting scissors; the eggshell was separated from egg content, the egg content were homogenized into a 50 mL beaker, (10 g) of the homogenized samples were weighed accurately in a tarred silica dish. After that the samples were dried at 120°C in a laboratory oven. These dishes were then placed in the muffle furnace at ambient temperature and slowly raised temperature to 450°C at a rate of 50°C/h. The samples were ignited in a Muffle furnace at 450°C for at least 8 hrs. Precaution was taken to avoid losses by volatilization of elements. After cooling the dishes of the samples were removed from furnace. Then samples were digested in desired amount of 50% nitric acid on hot plate. After that the samples were filtrated into a 100 ml volumetric flask using Whatman No. 44 filter paper and washed the residue.

Concentration of metals were determined and measured by an atomic absorption Spectrophotometer according to the AOAC, (2005) and calculated against a standard curve. Each analysis was carried in duplicate, standard and blank samples were analyzed every 20 sample. All concentration was expressed in µg kg^{-1} on dry weight basis.

Standard preparation

Metal standard solution was prepared for calibration of the instrument for each element being determined. All samples were prepared by the chemicals of analytical grade with distilled water. (1) Lead Standard solution–1mg / ml. 1.000 g Lead was dissolved in 7 ml conc HNO3 in 1 litre volumetric flask. Diluted to volume with water. (2) Cadmium Standard solution 1 mg / ml. 1.000 g cadmium was dissolved in 14 ml water and 7 ml conc HNO3 in 1 litre flask. Diluted to volume with water. The apparatus was adjusted at wave lengths of 217.0 nm for lead and 228.8 nm for cadmium.

Analysis of samples

Atomic Absorption Spectrophotometer was setting up with flame condition and absorbance was optimized for the analyses. Then blanks (deionized water), standards, sample blank and samples were aspirated into the flame in AAS. The calibration curves obtained for concentration vs. absorbance.

For potential health risk assessment, in the first step human health risks, posed by chronic exposure to heavy metals, were evaluated in accordance with Equation (1) (Guo et al., 2016).

Estimated daily intake (EDI) of heavy metal contaminants through egg consumption depends on heavy metal concentrations in the egg content, daily egg consumption, and consumer body weight, which is obtained using the following formula:

$$\text{EDI (mg)} = \frac{C \times 1Rd \ \times Cfactor}{bw} \quad \text{Equation (1)}$$

Where,

C is a heavy metal concentration (mg kg wet weight) in the egg content, *IRd* is a daily egg intake (11.64 g per day in 2016 Annual Per Capita Consumption of egg was 94 eggs/ capita/ year or (4.15 kg eggs /capita/year). (CAPMAS, 2018) in Egypt.), conversion factor (0.085) and bw is the average body weight of a normal adult was considered 65kg and for child was considered 27 kg.

In the second step, the Health Risk Index (HRI) for local population through egg consumption was assessed, using the Equation (2) (Guo et al., 2016).

$$HRI = \frac{EDI}{RfD} \qquad \text{Equation (2)}$$

Where,

EDI and RfD indicate the daily metal intake (mg) and reference dose of the metal (mg/kg/day), respectively. The oral reference doses were 0.0035 and 0.001 for Pb and Cd; respectively. When below 1, HRI means that the exposed population is assumed to be safe. Total HRI (THRI) of heavy metals for the eggs was calculated, according to Equation (3) (Guo et al., 2016).

THRI= HRI (toxicant 1) + HRI (toxicant 2)   Equation (3)

Statistical analysis

The statistical analysis was performed using SPSS (Statistical Package for Social Sciences) Software, Version 21. The descriptive statistics (mean values, standard error of mean) for values of egg content was analyzed. The level of significance for the differences was set at < 0.005 and < 0.001.

Results and discussion

In the present study, the toxic heavy metals Cadmium (Cd) and Lead (Pb) concentration were estimated, in whole hen's egg content. The concentrations of toxic heavy metals Cd and Pb were determined in two varieties of hen's egg (Balady and farm) collected from two wholesale markets of Alexandria and El-Behera Governorates; Egypt and summarized in Tables 1, 2 and 3. Results illustrated in Table 1 showed that the incidence of cadmium in Balady hen's eggs was 5 (16.67%) and in farm hen's eggs was 7 (23.33%) the concentration of Cd ranged from 0.1170 to 0.2620(µg kg^{-1}) with mean value of 0.1789 ± 0.0087 (µg kg^{-1}) from Alexandria Governorate (Table 1), and the incidence of Cd was 4 (13.33%) with concentration ranged from 0.10 to 0.19 (µg kg^{-1}) with mean value of 0.144 ± 0.0047 (µg kg^{-1}) in examined Balady hen's egg from El-Behera Governorate.

Table 1. Heavy metals Cadmium (µg kg^{-1}) concentration concentrations in eggs collected from Alexandria and El-Behera Governorates, Egypt.

Egg/district	No. of examined egg samples	Cadmium (µg kg^{-1}) concentration				
		No. of + ve samples		Min.	Max.	Mean ± SEM
		No.	%			
Alexandria Governorate	30	5	16.67	0.1170	0.2620	0.1789 ± 0.0087
Balady hen's egg						
Farm hen's egg	30	7	23.33	0.1160	0.6260	0. 2017 ± 0.0171
El Behera Governorate	30	4	13.33		0.19	0.144 ± 0.0047
Balady hen's egg						
Farm hen's egg	30	6	20	0.21	0.81	0.395 ± 0.0272
Total concentration of Cd (µg kg^{-1}) in all examined eggs	120	22/120	(18.33%)	0.10	0.81	0.23 ± 0.0122

Cd concentrations in Balady hen's egg from El- Behera Governorate were significantly lower than Balady hen's egg from Alexandria Governorate. From the finding presented in Table 1 it is clear that the incidence of Cd was 7(23.33%) and 6(20%) in farm hen's eggs from Alexandria and El-Behera Governorate; respectively. Cadmium (µg kg^{-1}) concentrations ranged from 0.1160 to 0.6260 (µg kg^{-1}) and 0.21 to 0.81(µg kg^{-1}) with mean value of 0.2017 ± 0.0171 and 0.395 ± 0.0272 (µg kg^{-1}); respectively in examined farm hen's eggs collected from Alexandria and El-Behera Governorates. The total concentration of Cd ranged from 0.1 to 0.81 (µg kg^{-1}) with mean value of 0.23 0.0122 (µg kg^{-1}) in all examined eggs.

The differences in Cd concentration between the farms hen's eggs and Balady hens egg were found to be statistically significant ($p<0.001$); The concentrations of Cd from examined farm hen's egg from El-Behera were significantly higher than those from Alexandria farm hen's egg. The level of Cd concentration was highest in Farm hen's eggs from El-Behera Governorate. The result of Cd contents in egg samples obtained in this study were lower than those obtained by Aliu et al. (2021) they reported (0.29–35.42 μg kg^{-1}) with mean value of (2.18±2.57 μg kg^{-1}) in eggs from Kosovo and higher than Saad Eldin and Raslan (2018) they reported that the residual concentrations of Cd was (0.18 ± 0.02) in eggs from Sharkia Governorate, Egypt and Kabir and Bhuyan (2019) they reported that the cadmium concentrations was recorded below the detection limit (ND (<0.003) in hens egg. Toxic elements such as Pb and Cd are known to be toxic and have maximum limits by European Commission in some foods but not for eggs (European Commission Regulation, EC No 1881, 2006).

Regarding the permissible limits of Cd it was clear that from results presented in Table 3, the mean concentrations of Cd in the entire examined hen's eggs were (0.23 ± 0.0122 μg kg^{-1}) which was below the maximum acceptable limits (0.05 mg kg^{-1}) in food recommended by EU, 2006); Lower findings were reported by Saad El Din and Raslan (2018), the study recorded the mean Cd concentrations in the examined egg samples were 0.18 ± 0.02, 0.09 ± 0.01 ppm in balady hen eggs. Lower results also were reported by Korish and Attia (2020); they reported undetectable level of Cadmium in chicken eggs from Saudi Arabia. Cd had been linked to skeletal damage (Jarup, 2003), and Cd and Pb are known to harm the reproductive system and embryonic development.

Table 2. Heavy metal (Lead, μg/kg^{-1}) concentration concentrations in eggs collected from Alexandria and El-Behera Governorates, Egypt.

Egg/district	No. of examined egg samples	Lead (μg/kg^{-1}) concentration				
		No. of + ve samples		Min	Max	Mean ± SEM
		No.	%			
Alexandria Governorate Balady hen's egg	30	12	40	38.00	281	106.43± 11.24
Farm hen's egg	30	17	56.67	112.00	741	303.03± 42.055
El Behera Governorate Balady hen's egg	30	6	20	19.00	55.0000	31.07± 2.16
Farm hen's egg	30	10	33.33	20.00	88.00	53.27± 3.43
Total concentration of lead (μg/kg^{-1}) in all examined eggs	120	45/120	(37.5%)	19.0	741	123.45 ± 14.597

Table 3. Compliance of examined eggs samples to maximum permissible limits of toxic metals in food.

Element	No of examined samples	Positive samples No (%)	Min.	Max.	Mean ± SEM (μg/kg^{-1})	Permissible limit	Over permissible limits No. (%)
Cadmium	120	22/120 (18.33%)	0.10	0.81	0.23 ± 0.0122	0.05(mg/ kg) According to EC, 2006.	Zero
Lead	120	45/120 (37.5%)	19.0	741	123.45 ± 14.597	0.1(mg/ kg) According to EC, 2006.	7/120 (5.83%)

Chicken eggs may pose risks due to contamination with heavy metals from the environment or through the food chain. Chicken exposure to heavy metals is due to ingestion of repeated small doses of such chemicals via feed and water. The major component of chicken feed as grains (maize, soybean and wheat) which are the main component of feed could also accumulated metals from contaminated soil depended on location. However food is the primary source of cadmium exposure, the main source of Cd contamination can be attributed to the use of chemical pesticides to cultivate poultry food as well as poultry medicines, containing cadmium and its adverse health effects occur in the form of kidney damage but possibly also bone effects and fracture. The concentration of the heavy metals Lead in examined hen's eggs are presented in Table 2, it is clear that 12 out of 30 (40%) Balady hen's eggs from Alexandria Governorate were found positive for lead with concentration ranged from 38 to 281 (μg kg^{-1}) with mean value of 106.43±11.24 (μg kg^{-1}). While in the analyzed Balady hen's egg samples from El-Behera Governorate the incidence of positive samples was 6/30 (20%) and the concentration of Lead was ranged from 19 to 55 (µg kg^{-1}) with mean value of 31.07± 2.16 (µg kg^{-1}). Lead concentrations were found higher significantly in Balady hen's eggs from Alexandria than Balady hen's eggs from El-Behera Governorate. The little variation in the metal content of egg bring at different location, feed appeared to be the major contributor to the amount of metals in eggs. These results also may explain the differences in bioaccumulation of the metal content in the eggs laid by hens from industrialized and agricultural regions (Dobrzañski, et al., 2004). Regarding the Pb element from farm hen's egg, results presented in Table 2 showed that the incidence of positive samples from Alexandria Governorate was 17 /30 (56.67 %); the lead concentration in farm eggs ranged from 112 to 741 (µg $kg^{-1)}$ with mean value of 303.03 ± 42.0.055 (µg kg^{-1}). While the lead incidence were 10/30 (33.3%) of examined farm hen's eggs from El-Behera Governorate and the minimum and maximum lead concentration were (20 and 88 µg kg^{-1}) with mean value of (53.27± 3.43 µg kg^{-1}). The incidence of Pb was 45/120 (37.5%) for all examined hen's eggs. The mean concentrations of Pb in all examined egg samples were (123.45 ± 14.59 µg kg^{-1}) (Table 2). The mean concentration of lead in farm hen's eggs from Alexandria was found significantly higher than mean concentration of lead from El-Behera Governorate. The results of Pb content in all examined hen's eggs were found highest in concentration in farm hen's eggs from Alexandria. Higher findings were obtained by Saad Eldin, and Raslan (2018) they reported that the residual concentrations of lead exceeded the maximum permissible limits set by (FAO)/(WHO) in all the examined Balady hen's eggs (0.34± 0.03 ppm/wet weight); from Egypt. Lower results were obtained by Korish and Attia (2020) they reported undetectable level of lead in chicken eggs from Saudi Arabia.; and Faryabi et al. (2021) they reported a concentration of 0.1163 mg kg^{-1} of lead in eggs from Qom, Iran. The mean concentrations of Pb in all examined eggs the present study were higher than those reported by Aliu et al. (2021) they recorded lead concentration range 0.04–1.41 µg $kg^{-1}$with mean value of (32.92 ± 23.18 µg kg^{-1}). The mean concentration of Pb in eggs from farms was significantly greater than the averages of Balady hens eggs (p<0.001). There were significant differences among the concentration of the two (Cd and Pb) metals included in the study between the egg samples from farms and Balady hen's eggs were observed. Pb concentration was the highest. The high Pb concentration in samples from farm hens can be explained due to the presence of the large industrialization in this region, which is also considered to be the main source of environmental pollution. EFSA (2012) reported that the environmental pollutants such as toxic element detected in free-range hen eggs that pasture outdoors are more exposed to environmental contamination were higher than those detected in battery-reared hen eggs. The levels of lead in eggs were significantly different ($P \leq 0.05$) among the two regions. The mean values of Pb concentration were near the permissible limit (0.1 mg kg^{-1}) in Balady eggs form Alexandria (0.106 mg kg^{-1}) and lower than the permissible limit for Balady eggs (0.031 mg kg^{-1}) and farm hens eggs from El-Behera (0.053 mg kg^{-1}) Governorates and exceeded the permissible limit in farm hens egg (0.303 mg kg^{-1}) from Alexandria. The variation may be resulted from feeding behavior and diverse capability for birds to ingest soil and grass. Researchers also found relationship between the levels of heavy metals in chicken and their feed like plant and insect. Hens' feed (water, additives, etc.) and husbandry system are the main influencing factors governing toxic element levels in eggs. These metals can pass to the egg representing a risk factor in humans who consume contaminated hen's table eggs.

Regarding the permissible limits of Pb, it was clear that from results presented in Table 3, 7 out of 120 examined hen's egg samples (5.83%) exceeded the maximum acceptable limits in food (0.1 mg kg^{-1}) recommended by EU, 2006 (Table 3). A significant difference ($P \leq 0.05$) was observed between the mean concentration (0.123 mg kg^{-1}) of the studied samples and the international standard lead level in eggs (0.1 mg kg^{-1}). Approximately 40% uptake of lead from egg is reported in children Khan et al. (2016). High lead concentration in food has been reported to be associated with cardiovascular, renal, nervous, and skeletal-system diseases (Jarup, 2003). The major sources of metal contamination in commercially produced eggs include contamination in the feed, water as well as contaminations in the environment. Poultry ration in many cases contain bone or fish meals, which are considered major sources of feed contamination with metals. Lead ingested by chicken through contaminated feed is deposited in bones; soft tissue and eggs, so the contaminated egg represents a potential public health hazard. Table 4 showed the estimated daily intake (mg kg^{-1} bw. day) of Cd and Pb heavy metals by Egyptian adults and children through consumption of hen's egg compared against (JECFA) maximum permitted daily dietary allowance per person and recommended daily allowance (RDA) for adults and children. The maximum provisional tolerance daily intake of Pb is based on the provisional tolerable weekly intake (PTWI) the recommended daily allowance for adults JECFA, (2009) and the maximum provisional tolerance daily intake of Cd is based on the provisional tolerable monthly intake (PTMI) of JECFA (Commission 2013).

Table 4. The estimated daily intake (EDI, mg/kg bw day) of Egyptian adults and children according to mean weight of Cd and Pb toxic heavy metals in total of the examined eggs compared to the recommended daily allowance set by JECFA for adults and children.

Toxic heavy metal	RDA (mg/day /person)	MTDI (µg/kg bw/day)	MTMI mg/kg bw.	EDI (mg /kg^{-1} bw day^{-1})	
				Adult	Child
Cd	0.06	7	0.025 mg/kg bw/month JECFA (Commission 2013)	0.000035	0.000084
Pb	0.21	The JECFA PTDI of Pb is 3.6 µg/kg bw/day (WHO, 2005). (0.0036 mg/kg)	--	0.0019	0.0045

RDA: Recommended daily dietary allowance, MTDI: Maximum tolerable daily intake;
MTMI: Maximum tolerable monthly intake

The EDI (mg kg^{-1} bw day^{-1}) of Cadmium for adult was 0.000035; and 0.000084 for children which was lower than the PTMI (0.025 mg kg^{-1} Bw/month) JECFA (commission 2013) indicating no health risk for Cd through egg consumption among Egyptian consumers. The EDI (mg kg^{-1} bw day^{-1}) of Pb for adult was 00.0019 and 0.0045 (mg kg^{-1} bw day^{-1}) for lead for adult and child consumers; respectively. The JECFA (WHO, 2005) PTDI of Pb (0.0036 mg kg^{-1}). Therefore, it can be concluded that people might face no potential significant health risk through mere consumption of the analyzed eggs, under the current consumption rate except for pb in child consumer as it exceeded the JECFA PTDI of Pb (0.0036 mg kg^{-1} bw day^{-1}). Chronic lead exposure causes developmental abnormalities, deficits in intelligence quotient, neurotoxicity in infants, constipation, colic and anaemia and also can damage intellectual performance, resulting in reduced cognitive development in children. Young children are particularly vulnerable to lead poisoning because they absorb 4–5 times as much ingested lead as adults from a given source (WHO, 2021) no safe level of lead exposure has yet been identified for children's health. Excess lead in food can cause serious damage to the brain, kidneys, nervous system and red blood cells in human's toxic elements can be very harmful even at low concentration when ingested over a long time period.

The Health Risk Index results were showed in Table 5 assuming the weekly consumption rate of three eggs per person revealed that the Health Risk Index (HRI) for Cd for child was 0.0035 and was 0.00842 for adult human consuming hen's eggs was within the safe limits as the value (HRI < 1) while the HRI for Pb (0.5368) was within the safe limits for adult consumers (HRI < 1) and unsafe for children (1.2878) as it (HRI >1). Results also showed that the total HRI was safe (0.5403) for adult consumers and unsafe (1.2962) for children (HRI >1). The continuous intake of heavy metals through food at unsafe levels could have adverse effects in the terms of disrupting many biological and biochemical processes in humans, especially in children. Metals can bio accumulates over time to reach toxic levels, which can cause decreases in reproductive success and lowered survival (Burger, 1999). Lead is a cumulative toxicant that affects multiple body systems and is particularly harmful to young children. Drinking water delivered through lead pipes or pipes joined with lead solder may contain lead (WHO, 2021). Once lead enters the body, it is distributed to organs such as the brain, kidneys, liver and bones. At lower levels of exposure that causes no obvious symptoms, lead can affect children's brain development, causes anaemia, hypertension, renal impairment, immunotoxicity and toxicity to the reproductive organs. A regular monitoring of heavy metals in hen's eggs is recommended at an appropriate frequency to establish the true contribution of eggs to the dietary intake of heavy metals to avoid adverse toxic effects for human consumption.

Table 5. Health Risk Index (HRI) for local population through hen's egg consumption.

	Cd	Health risk	Pb	Health risk	Total HRI (THRI)
Adult	0.0035	Safe	0.5368	Safe	0.5403
Child	0.00842	Safe	1.2878	Unsafe	1.2962

Conclusions

It is concluded that cadmium was found to be in the normal range reported in the literature and lead health risk through egg consumption is within safe limits for adult consumers but exceeded the permissible limit for children indicating health hazard for lead through egg consumption among Egyptian child consumers. However, the presence of toxic metals, Cd and Pb was higher in poultry farm eggs than Balady hen's eggs. The nutritional importance and high consumption of eggs among households necessitate a more careful monitoring of lead concentrations to meet public health requirements. Environment pollution with heavy metals lead to an increased attention in metal contamination of food and amongst them eggs which represent an essential part of human's diet particularly children.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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